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14. ABSTRACT  Full length recombinant human lubricin (rhPRG4) maximally reduced friction between apposed explants of bovine articular cartilage. Secretion levels exceeded 1 mg/ml when expressed by transfected CHO-M cells. This level of expression is commercially scalable and will thus enable production of a GMP protein for clinical use. A purification bioprocess utilizing anion exchange, hydroxyapatite and a hydrophobic exchange media resins achieves a high level of purity. Explants of bovine articular cartilage cultured and incubated with IL-1 followed by friction testing showed higher friction than explants not receiving IL-1. The rhPRG4 restored low friction and qualitatively restored native PRG4 expression as determined by immunohistochemistry. Cartilage from the femoral condyle shows variations in IL-1 induced elevated friction. Explants from the weight bearing region show a significant response to exogenous rhPRG4 in friction reduction from this simulated inflammatory environment. A large animal trial is now planned to test pre-GMP equivalent rhPRG4 in restoring chondroprotection in the ACL-injured joint.					
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## INTRODUCTION

We have produced recombinant human lubricin (rhPRG4), which we hypothesized would provide disease modifying relief to patients at risk for post-traumatic OA of the knee. The epidemiologic civilian experience indicates that 18% of the total OA burden is of traumatic origin. Among military servicemen and women, acute knee injuries comprise almost 5% of all reported injuries. Intra-articular (IA) injections of recombinant lubricin in pre-clinical rodent models show a chondroprotective effect upon cartilage structure and chondrocyte metabolism. Lubricin is a mucinous glycoprotein which we show can be manufactured by CHO-M cells. The point of this work was also to establish that the production of this recombinant human protein is scalable to levels required for the treatment of patients. We have identified a bioprocess for purification and have ascertained that the recombinant protein is active in an *in vitro* cartilage explant bearing system. We identified a candidate recombinant lubricin that possesses scalability and maximal chondroprotective bioactivity in the form of low friction. We also reproduced this result in a cartilage explant stimulated with IL-1 to induce cartilage damage. We have also tested this rhPRG4 for its ability to diminish caspase-3 activity in chondrocytes which confirmed its friction-reducing activity *in vitro*. That candidate underwent clonal expansion and enough lubricin was purified to enable a trial in porcine to determine if intra-articular injection of lubricin minimizes cartilage damage following ACL transection, and after ACL transection followed by ACL reconstruction, the current gold standard in which the torn ligament is replaced by an autograft or allograft. Previous work with this model has shown that ACL reconstruction does not minimize cartilage loss. In parallel with this project, we also performed another study in porcine following meniscal injury, which was funded by a Phase 2 STTR (1R42AR057276 PI: Jay) using the same rhPRG4 also produced under pre-GMP conditions. The meniscal injury model involved destabilization of the medial menisco-tibial ligament. We are disclosing results from both studies in this progress report as they are related but completely separate efforts. It should also be noted that the data from the ACL reconstruction model are currently being analyzed and compared.

## KEYWORDS

Chondrocytes, arthritis, osteoarthritis, lubricin, PRG4, lubrication

## ACCOMPLISHMENTS

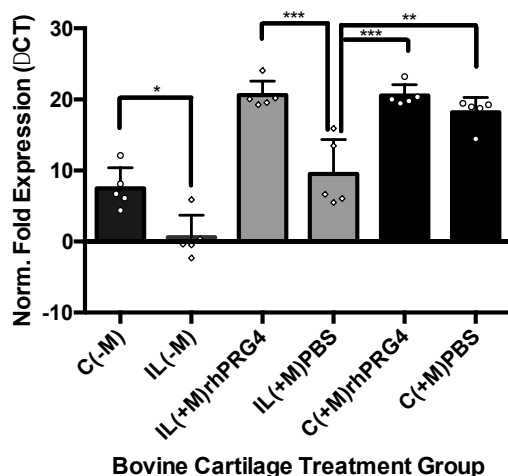
**AIM 1:** Designing and implementing a laboratory grade production of therapeutic lubricin candidates which can ultimately be replicated by a well-established turnkey partner for protein production who possess the capability to manufacture a large glycoprotein. **Rationale:** A potentially therapeutic lubricin must be identified, structurally defined and its mode of expression determined, which will directly impact its likelihood of manufacture.

COMPLETED. A scalable method of rhPRG4 production has been created utilizing CHO-M cells detailed in the last progress report. We also determined that endotoxin levels are acceptably low ~0.1 EU/ml when tested by the QCL-1000™ Chromogenic LAL Assay (Lonza) and the CHO-M cells of origin tested negative for rodent viruses by Charles River (Andover, MA).

**AIM 2:** Identify a candidate lubricin from Aim 1, which has maximal chondroprotection in an *in vitro* cartilage bearing model, which complements scalability, and a low cost-of-goods. **Rationale:** Identification of a lubricin candidate, which is destined for GMP or GMP-like production, should demonstrate a reproducible ability to reduce friction *in vitro* and promote chondrocyte survival. As pointed out in the last progress report, we concluded that the full-length rhPRG4 construct is the best candidate from both a chondroprotective and scalability vantage point. We collected additional data in cartilage explants cultured for 7 days in cell culture media containing IL-1 $\alpha$  and further supported this conclusion. In addition, we also performed RT-PCR experiments to determine if expression of PRG4 was downregulated at the mRNA level in this inflammatory milieu, and also tested if a well lubricated bearing could restore native PRG4 expression. This was necessary to settle a debate in the literature over whether inflammation up- or down regulates PRG4 expression.

**RESULTS:** IL-1 $\alpha$  stimulated bovine cartilage explants showed an increase in static COF from  $0.15 \pm 0.052$  to  $0.21 \pm 0.059$ , ( $p=0.05$ ) when lubricated with phosphate buffered saline. Dynamic and static COF in IL-1 $\alpha$  stimulated explants lubricated with recombinant human PRG4 (rhPRG4) compared to those lubricated with saline decreased from  $0.064 \pm 0.031$  to  $0.026 \pm 0.018$ , ( $p=0.002$ ) and  $0.21 \pm 0.059$  to  $0.14 \pm 0.065$ , ( $p=0.02$ ) respectively. PRG4 expression was decreased 7 fold under IL-1 $\alpha$  stimulation in non-mechanically stimulated explants compared to IL-1 $\alpha$  free controls ( $p=0.01$ ). However, use of rhPRG4 as the lubricant significantly increased native PRG4 expression to a nominal level compared to phosphate buffered saline in IL-1 $\alpha$  treated explants ( $p<0.0001$ ) (Fig 1). All mechanically stimulated explants expressed significantly more PRG4 than their non-mechanically stimulated counterparts. Additionally, there was a significant increase in PRG4 mRNA for IL(+M)rhPRG4 explants compared to IL(+M)PBS ( $p<0.0001$ , Figure 1). There is also a significant increase in PRG4 mRNA for C(+M)rhPRG4 and C(+M)PBS compared to IL(+M)PBS ( $p<0.0001$ ,  $p=0.001$ , Fig 1). *These data suggest that rhPRG4 is able to restore PRG4 expression in cartilage explants, when mechanically loaded and oscillated, back to normal control levels in simulated inflammatory conditions.*

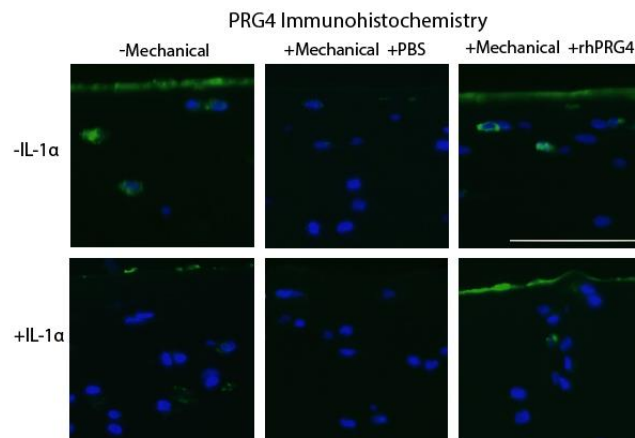
This is further supported qualitatively in Figure 2 where IL-1:rhPRG4 explants displayed a marked



**Figure 1: Normalized PRG4 mRNA Expression Across Cartilage Explant Groups.** PRG4 mRNA for mechanically (+M) and non-mechanically stimulated (-M) bovine cartilage explants cultured with IL-1 $\alpha$  supplemented media (IL, gray) or control media (C, black). Either phosphate buffered saline (PBS) or recombinant PRG4 (rhPRG4) was used as a lubricant for +M explants. There is a significant decrease in PRG4 mRNA for IL(-M) explants as compared to C(-M) explants, (\* $p=0.01$ ). There is a significant increase in PRG4 mRNA for IL(+M)rhPRG4 explants compared to IL(+M)PBS (\*\*\* $p<0.0001$ ). There is also a significant decrease in PRG4 mRNA for IL(+M)PBS compared to C(+M)rhPRG4 and C(+M)PBS respectively (\*\*\* $p<0.0001$ , \*\* $p=0.001$ ).

increase in PRG4 accumulation compared to IL-1 $\alpha$  treated, non-mechanically stimulated, explant controls. The increase in PRG4 accumulation for IL-1:rhPRG4 explants was significantly different than the nonexistent PRG4 accumulation seen in IL-1:PBS explants.

**Figure 2: PRG4 Immunohistochemistry.** Immunohistological detection of PRG4 by 9g3 (green) and cell nuclei via DAPI (blue) in non-mechanically stimulated (-Mechanical, -M) and mechanically stimulated (+Mechanical, +M) bovine cartilage explants cultured with IL-1 $\alpha$  (+IL-1 $\alpha$ , IL) or control media (-IL-1 $\alpha$ , C) is shown in six representative images. Either phosphate buffered saline (PBS) or recombinant PRG4 (rhPRG4) was used as a lubricant for +M explants. IL(-M) explants displayed decreased PRG4 compared to C(-M) explants. IL(+M)PBS explants displayed no change in PRG4 while IL(+M)rhPRG4 explants displayed increased PRG4 on the surface comparable to C(-M) explants and C(+M)rhPRG4 explants. C(+M)PBS explants displayed marked decrease in PRG4 compared to C(-M) and C(+M)rhPRG4 explants. Scale bar is 100 microns.

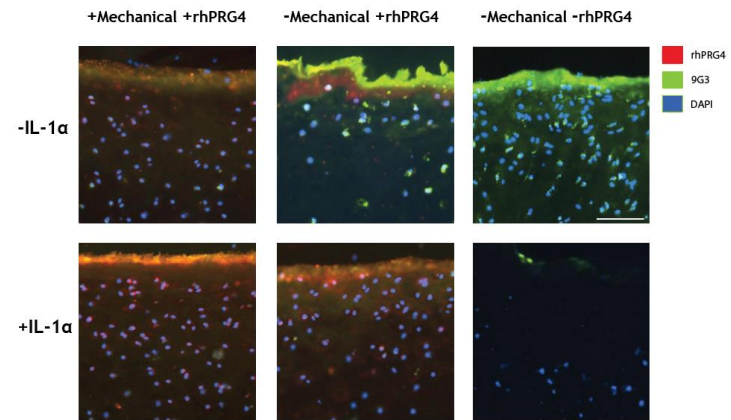


Rhodamine labeled rhPRG4 was used to assess the extent to which rhPRG4 attached and infiltrated the cartilage in IL(+M)rhPRG4 and C(+M)rhPRG4 explants. A greater amount of rhodamine labeled rhPRG4 was observed in the cartilage of IL(-M) explants compared to C(-M) explants (Figure 3). *Clearly the labeled rhPRG4 accumulates on the articular surface and also appeared to penetrate into the cartilage to some extent.* Merging of the green and red channels in the microscope digital camera also showed that the applied rhPRG4 and native PRG4, that was either pre-existing or nascently secreted, co-habitated on the surface. Explants treated with IL-1 $\alpha$  appeared to be populated by more labelled rhPRG4 than native PRG4, supporting similar observations in Figs 1 and 2.

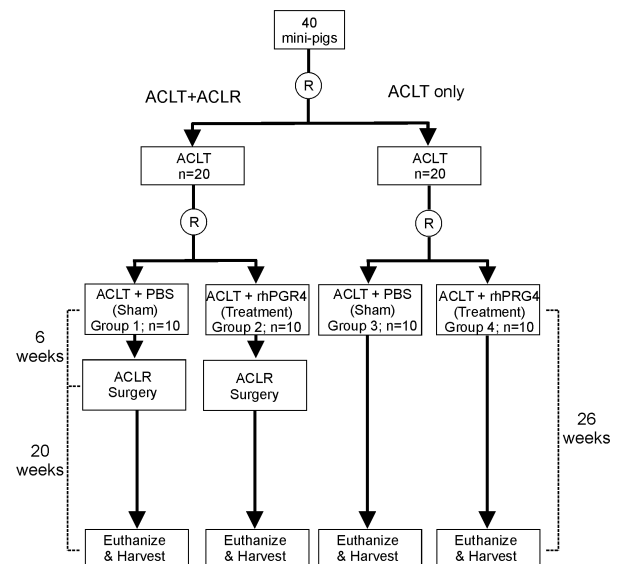
Thus, we have shown that full-length rhPRG4 is a potential therapeutic ex vivo in the prevention of OA following joint trauma, and in inflamed joints predisposed to OA based on other risk factors. The ability of rhPRG4 to diminish activated caspase-3 in chondrocytes in these osteochondral explant bearings is presently being analyzed.

**Figure 3: Immunohistochemistry of PRG4 in the Presence of Exogenous Rhodamine Labeled rhPRG4.**

Representative images depicting exogenous (red) and a combination of exogenous and endogenous PRG4 for IL-1 $\alpha$  treated (+IL-1 $\alpha$ ) and control (-IL-1 $\alpha$ ) explants with (+rhPRG4) or without (-rhPRG4) rhodamine labeled rhPRG4. Green indicates detection of all PRG4 present by 9g3 (whether endogenous or exogenous) and red indicates exogenous rhodamine labeled rhPRG4 and counter staining of cell nuclei with DAPI is blue. Samples lubricated with rhPRG4 were either mechanically stimulated (+Mechanical) or not mechanically stimulated (-Mechanical). IL-1 $\alpha$  stimulated explants without rhPRG4 exhibited little to no endogenous PRG4, while IL-1 $\alpha$  stimulated samples with rhPRG4 displayed large amounts of exogenous rhPRG4 penetrating into the cartilage superficial zone. IL-1 $\alpha$  stimulated samples appear to exhibit more exogenous rhPRG4 attachment than their control treated counterparts. This suggests that IL-1 $\alpha$  stimulation radically diminishes endogenous PRG4 while allowing for increased exogenous rhPRG4 attachment and penetration into the cartilage tissue. Scale bar is 100 microns.



**Figure 4: Experimental design schema**  
**ACLT +/- rhPRG4 treatment with 6 week delayed ACL reconstruction (ACLR) and**  
**ACLT +/- rhPRG4 treatment without ACLR.** The study of these *four* groups is completed however tissues are being decalcified for histology, and CTX-II and IL-1 $\beta$  assays are in process.



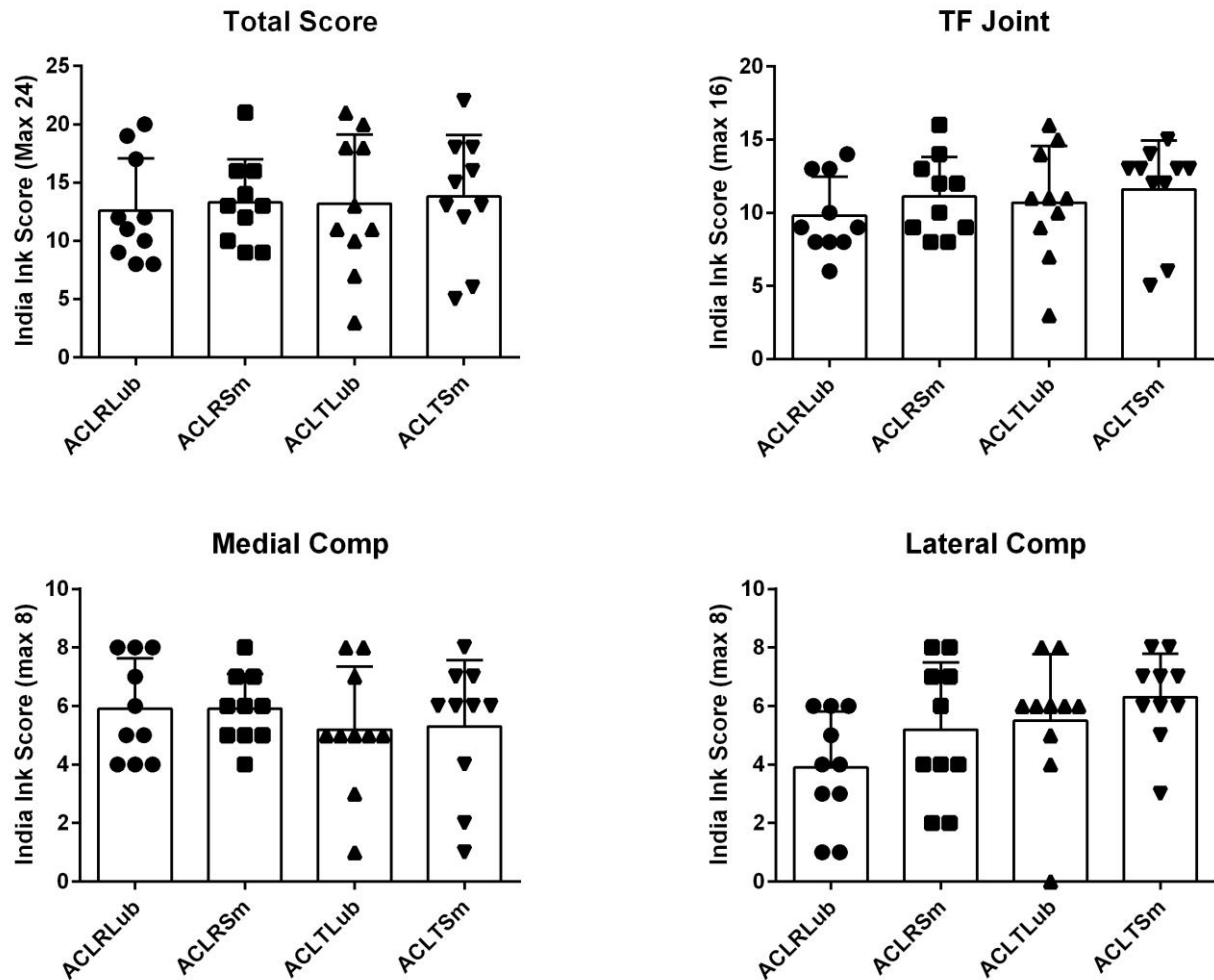
**Aim 3:** In this study we utilized a large animal model to evaluate whether intra-articular injection of rhPRG4 following ACL transection preserved cartilage after delayed ACL reconstruction (ACLR) surgery. **Rationale:** Large animal studies are a FDA required prelude to human studies. The porcine diarthrodial joint was selected as it is biomechanically more similar to human joints than those of other animal models.

Porcine were assigned randomly to one of the four treatments using a uniform random number generator (Fig 4). A 50kg animal receiving rhPRG4 received 4 mg of sterile rhPRG4 delivered intra-articularly in a 3ml PBS carrier **once a week for 3 weeks** (12 mg) which is 240 µg/kg/animal. These injections began on post-ACLT day 7. Each animal in the standard ACLR groups (Groups 1 & 2) had the ACL transected followed by ACLR 6 weeks later using bone-patellar tendon-bone (BPTB) allograft, which was obtained from the central third of the patellar tendon from donor animals. Each animal will undergo the identical surgical procedure. At 6 months, the 10 animals in each group were anesthetized and underwent clinical examination for joint laxity measurement, after which they were euthanized so that the limbs could be harvested (disarticulated at the hip). The limbs were all stored at 4°C immediately after harvest and until dissection at the Biomechanics lab at RIH Coro West 4<sup>th</sup> floor.

The femur and tibia were separated and India ink staining of the cartilage surfaces of the femur and tibia were performed by brushing a 10% solution of India ink in PBS over the joint surfaces, allowing it to sit for 60 seconds and then washing in tap water to remove excess ink. The joints were then sequentially photographed to document the trochlear surface, weight bearing surfaces of the femoral condyles and posterior femoral condyles, and the tibial plateau. All photographs were taken while minimizing light contamination. Photographs will then be assembled for each knee. Three graders, blinded to the identity of the photographs marked on a template if ink uptake was visible in any of the following 11 locations: Trochlea, medial femoral condyle (MFC) near the notch, lateral femoral condyle (LFC) near the notch, MFC weight bearing surface, LFC weight bearing surface, posterior MFC, posterior and central LFC, posterior LFC adjacent to the notch, central medial tibial plateau (MTP), central lateral tibial plateau (LTP), and LTP near the tibial spine.

**PRELIMINARY RESULTS:** Forty Yucatan minipigs (20 castrated male, 20 female, weight 54.1±3.9 kg, age 15.9±0.9 months) underwent ACL transection or ACL reconstruction surgery, using age and sex-matched patellar tendon grafts harvested from Yucatan donors (weight 54.8±4.5 kg, age 17.8±1.2 mo). Seven, 14 and 21 days after surgery, the animals received intra-articular injections (3 ml volume per injection) of saline (N=9) or recombinant human lubricin (rhPRG4, 1.33 mg/ml, N=10) (3), depending on the randomized group assignment. All solutions contained 0.1% Tween used as a stabilizer in the rhPRG4 test article. Injections were performed under ultrasound guidance. Twenty-six weeks following surgery, the hind limbs were harvested and the knees were dissected to evaluate joint integrity, and synovial fluid, blood serum and urine were collected to evaluate biomarkers.

India ink scores showed scatter in all groups when all scores were summed for all cartilage surfaces within each joint. Analysis of the isolated tibiofemoral scores also showed scatter and did not demonstrate statistical differences between treatment groups. However upon separating the data into medial and lateral compartments we noted that the ACL Reconstructed group treated with rhPRG4 showed a significant ( $p = 0.04$ ) reduction in India ink staining (Fig 5) in the lateral compartment of the tibio-femoral (TF) joint. In each of these specimens the worst lesion was scored regardless of its location on the cartilage surface. In the ACLT model in the porcine we observed that many of the lesions were located not in the central area of the main weight bearing area but in ectopic locations in the margins of the tibial or femoral condyle. Thus we plan to re-score in a blinded fashion all of the cartilage surfaces with the intent of focusing on the main weight bearing area of the tibial and femoral condyle as the cartilage in normal joints does not appear to be normal (for example, the normal cartilage at the interface between the tibiofemoral and patellofemoral joints appears damaged because it is not under load). It is possible that our emphasis on identifying and measuring the worst lesions may obscure the chondroprotective effects that are not immediately apparent in the data. Tissue samples are also in the process of being decalcified prior to histological processing which will require more time. In addition, synovial fluid and serum are being assayed for PRG4 levels, and both fluids are being assayed for IL-1 levels. Data analyses of quadruped gait symmetry across all porcine before and after surgery across all 4 groups are also in progress.



**Figure 5: ACLT/ACLR study mean India ink scores.** Three graders, blinded to the identity of the photographed cartilage surfaces noted if ink uptake was visible in any of the following 11 locations: Trochlea, medial femoral condyle (MFC) near the notch, lateral femoral condyle (LFC) near the notch, MFC weight bearing surface, LFC weight bearing surface, posterior MFC, posterior and central LFC, posterior LFC adjacent to the notch, central medial tibial plateau (MTP), central lateral tibial plateau (LTP), and LTP near the tibial spine of the surgical limbs. No significant differences in India ink scores were noted for the whole joint (Total score) and the isolated tibio-femoral (TF) joint across the 4 groups. However upon comparing the medial and lateral joint compartments within the TF joint, a significant decrease in India ink staining was noted the ACL reconstructed group that received rhPRG4 (ACLRLub) following the initial surgery to transect the ACL. There were no differences among porcine that had the ACL reconstructed that received saline vehicle (ACLRSm), or the ACL transected animals that received either rhPRG4 (ACLTLub) or saline (ACLTSm). This analysis is preliminary and will require a sub analysis limited to the weight bearing condylar surfaces.



## **Related Work in the Porcine Using the Destabilization of the Medial Meniscus (DMM) Model (Supported by NIH 1R42AR057276)**

Aims 1 and 2 of this CDMRP Award enabled progress in another porcine study that was being conducted contemporaneously. In this study, we determined the ability of recombinant human lubricin (rhPRG4) to preserve the tibiofemoral joint cartilage after surgical destabilization of the medial meniscus (DMM), in a porcine model. We hypothesized that the macroscopic cartilage integrity score of surgical joints, and the serum and synovial fluid markers of inflammation and cartilage damage of joints treated with injection of rhPRG4 or rhPRG4+hyaluronan (rhPRG4+HA) would be significantly less than those treated with placebo injection (saline containing 0.1% Tween) 26 weeks following DMM. We also hypothesized that injection with rhPRG4+HA would result in improved results compared to rhPRG4 alone.

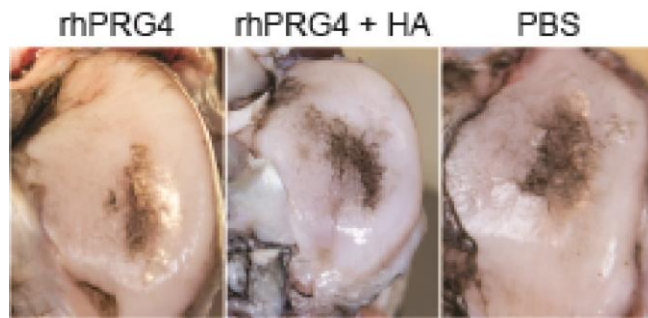
**METHODS:** 30 Yucatan minipigs (15 castrated male, 15 female, weight  $55.5 \pm 2.1$  Kg) underwent DMM by excising a 5 mm full-thickness section of the anterior meniscal horn attached to the menisco-tibial ligament. Note that one animal died at the time of surgery and was not included in the study. Seven, 14 and 21 days after surgery, the animals received intra-articular injections (3 ml volume per injection) of PBS (N=9), recombinant human lubricin (rhPRG4, 3 mg/ml, N=10) (3), or rhPRG4+HA (3 mg/ml rhPRG4 and 3 mg/ml HA, 700 kDa, R&D systems, Minneapolis, MN, N=10), depending on the randomized group assignment. All solutions contained 0.1% Tween. Injections were performed under ultrasound guidance. Twenty-six weeks following surgery, the hind limbs were harvested and the knees were dissected to evaluate joint integrity.

The medial tibial plateau and femoral condyle of the surgical limbs were stained with India ink and photographed for independent scoring by two blinded reviewers. Scores ranged from 0 (smooth surface) to 4 (lesion with more than 10% exposed bone). Lesion lengths and widths were measured using calipers, and lesion areas were approximated as an ellipse based on these measurements. Blood serum and urine were collected for all animals at the time of harvest. Synovial fluid was directly collected via syringe from the harvested joint, without lavage, for 23/29 animals. PRG4 concentration was measured using inhibition ELISA with 9G3 on synovial fluid samples. IL-1 $\beta$  concentration was determined in synovial fluid and serum using ELISA. Serum and urine CTXII was measured using the Cartilaps kit, and the urine values were normalized against urinary creatinine.

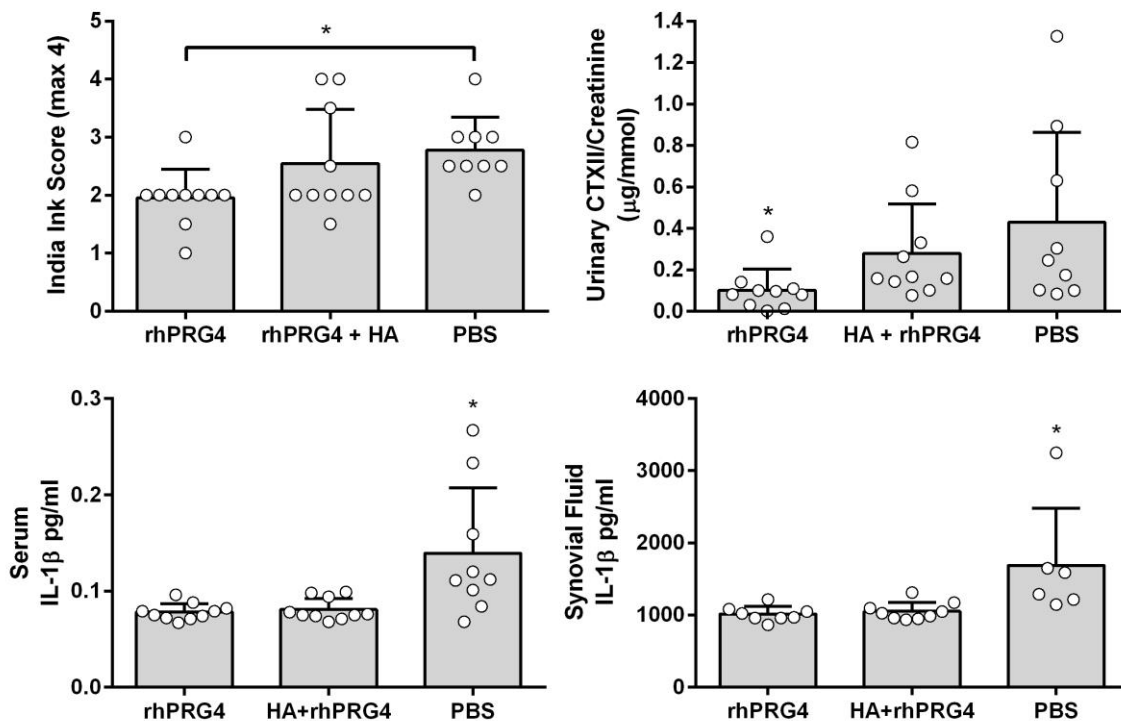
Comparisons between groups were performed using a Kruskal-Wallis test ( $\alpha=0.05$ ) and Dunn's multiple comparison tests. The intra class correlation (ICC) was determined to evaluate the reliability of the scoring between examiners (ICC = 0.75). All experiments were approved by the RI Hospital and Brown University Institutional Animal Care and Use Committees.

**RESULTS:** All subjects showed degenerative changes in the cartilage of the medial tibial plateau (MTP) of the surgically treated knee (Fig 6). Subjects receiving rhPRG4 treatment had significantly lower scores in the MTP compared to PBS control ( $p=0.01$ ) (Fig 7), but lesion area differences failed to reach significance between groups ( $p>0.05$ ). Lesions in the medial femoral condyle (MFC) were less severe compared to those in the tibial plateau, and did not significantly differ in score between groups ( $p>0.05$ ). MFC lesions were located in the anterior portion of the condyle, with some extending into the posterior region. Lesion size in the MTP and MFC did not significantly differ between groups, but lesion severity in the PBS group was high for large lesions, indicating an accelerated disease state. Synovial PRG4 concentration was significantly higher in rhPRG4 treated knees compared to PBS treated knees ( $p=0.02$ ). Serum and synovial IL-1 $\beta$  was significantly lower in knees treated with rhPRG4 or rhPRG4+HA compared to PBS ( $p<0.05$ ). Urinary CTXII was significantly lower in rhPRG4

treated limbs compared to other groups ( $p<0.047$  and  $p=0.02$  for rhPRG4+HA and PBS, respectively) (Fig 7).



**Figure 6: Median value India ink stained medial tibial plateaus (MTP) from the DMM study.** Surgical and contra-lateral limbs from porcine that underwent destabilization of the medial menisci from each of the three treatment groups were stained with India ink. Scoring was conducted by two blinded reviewers and ranged from 0 (smooth surface) to 4 (lesion with more than 10% exposed bone).



**Figure 7: Comparison of the three treatment groups in the porcine DMM study.** India ink scores were significantly lower in the MTP. Notably, urinary CTX-II corrected for urine creatinine was significantly lower for porcine treated with rhPRG4 alone compared to vehicle only (PBS) or the combination of hyaluronate (HA) and rhPRG4. Both serum and SF IL-1 $\beta$  was also lower in those animals that received rhPRG4 with or without HA. The rhPRG4 dosing was conducted in the same manner as in the above ACLT/ACLR study.

## DISCUSSION:

The DMM model produced consistently located lesions in surgically treated tibiofemoral joints. We predict that release of the medial meniscus causes the structure to move outward,

increasing contact stress between the medial femoral condyle and medial tibial plateau. In addition, inflammatory factors, such as IL-1 $\beta$ , are present in response to the injury and have been shown to down-regulate endogenous PRG4 and subsequent lubrication. Our findings indicate that three weekly intraarticular injections of rhPRG4 attenuates cartilage damage in the MTP of damaged joints, and we hypothesize that this effect is achieved by the restoration of boundary lubrication and by anti-inflammatory activity. Interestingly, the rhPRG4+HA treatment failed to show significant improvements over PBS injection. We hypothesize that this effect may be attributed to the CD44-mediated anti-inflammatory effects of lubricin and HA, where both molecules have been shown to compete for the hyaluronan receptor CD44 (1).

Further analysis of tissues, including protein staining, chondrocyte apoptosis and histological analysis and scoring are in progress for both the ACLT/ACLR and DMM studies. Limitations of the ACLT/ACLR study include the surgical insult without repair, which mechanically promotes arthrosis. Also, while rhPRG4 injections were completed after 4 weeks, joint integrity was assessed after 26 weeks, which may explain why chondral damage was present in all treatment groups of both studies. Despite this limitation, the rhPRG4 injections slowed disease progression as noted in the meniscal injury model.

Presently the resulting data from the ACLT/ACLR model appears to be highly variable. This may be due to the fact that these assessments are ongoing or because the model is confounded by other variables that obscure the chondroprotective effects of the rhPRG4. For example, we have yet to look at the post-surgical joint laxity data as we remain fully blinded in the analysis thus far. In addition, serum and SF analyses are also in the process of being conducted. Histological analysis is delayed due to the lengthy decalcification process required for the probes to measure activation of caspase-3, the apoptosis marker.

## KEY RESEARCH ACCOMPLISHMENTS

- Rationalization that full-length rhPRG4 is a better lubricant than mucin domain truncated products
- Truncated products missing 3 glutamine residues in the center of the mucin domain significantly diminishes expression
- Expression of full-length rhPRG4 from CHO-M is possible and is a scalable production platform
- Full-length rhPRG4 as the selected construct for the large animal study in Aim 3 also reduced friction in IL-1 stimulated cartilage explants which partly recapitulates the environment of the post-traumatic joint
- In the porcine ACLT joint, based on India Ink staining alone, we observed that both ACLR and rhPRG4 treatment separately decreased India ink scores but not significantly. These analyses are still ongoing.
- However in the porcine ACLT joint, based on India ink staining alone, we observed that the *combination* of rhPRG4 treatment after the ACLT, followed by the reconstruction of the ACL, significantly decreased India ink score in the lateral knee joint compartment.
- In related work in porcine, simulating a meniscal injury followed by 3 weekly injections of rhPRG4- we observed a *robust* decrease in India ink score, urinary CTX-II and serum and synovial levels of IL-1.

## IMPACT

PRG4 is down regulated in inflammation and trauma, and can be partly restored using recombinant lubricin which in turn promotes chondroprotection. In the absence of adequate PRG4, less native PRG4 is expressed and chondrocytes die via apoptosis. Patients with either an ACL or meniscus injured knee joint are potential beneficiaries of this research. Further research in large animals, like porcine, is needed to test the equivalence of 3 weekly injections of rhPRG4 versus a single dose escalated intra-articular supplementation of rhPRG4. We also learned that hyaluronate, which is currently being used to treat osteoarthritis, is unnecessary in this form of treatment. The combination of rhPRG4 and HA did not enhance results in the traumatized joint, and in fact were no better than the placebo treatment alone. Efforts to utilize protein production platforms to manufacture rhPRG4 are a logical next step to ensure commercialization of rhPRG4. Future work will now also include demonstrating that rhPRG4 is necessary in providing chondroprotection following arthroscopic surgery for meniscal repair and in ACL reconstruction. These are logical extensions of the current work since these are clinical opportunities created by routine entry into the joint space where copious joint irrigation removes native PRG4 and leaves the cartilage surfaces vulnerable to friction induced damage.

## CHANGES/PROBLEMS

Preparation of rhPRG4 in Aim 2 did require an additional 2 months beyond our anticipated timeline. Thus Aim 3 began in May 2015 instead of the end of 2014 as originally proposed. While this will delay final publication of the results, we expect to complete the data analyses within the coming year.

## PRODUCTS

### Publications

1. Ludwig, T., Cowman, M., **Jay, GD.**, Schmidt, T. Effects of Concentration and Structure on Proteoglycan 4 Rheology and Interaction with Hyaluronan. *Biorheology*. 2015 Jan 1;51(6):409-22
2. Elsaid, K., Zhang, L., Shaman, Z., Patel, C., Schmidt, TA., **Jay, GD.** The Impact of Early Intra-Articular Administration of Interleukin-1 Receptor Antagonist on Lubricin Metabolism and Cartilage Degeneration in an Anterior Cruciate Ligament Transection Model. *Osteoarthritis Cartilage*. 2015 Jan;23(1):114-21.
3. Ai, M., Cui, Y., Sy, M., Lee, DM., Zhang, LX., Larson, KM., Kurek, KC., **Jay, GD.**, Warman, ML. Anti-Lubricin Monoclonal Antibodies Created Using Lubricin-Knockout Mice Immunodetect Lubricin in Several Species and in Patients with Healthy and Diseased Joints. *PLOS One*. 2015 Feb 2;10(2).[ PMID:25642942]
4. Al-Shari, A., Jamal, M., Zhang, L., Larson, K., Waller, K., Schmidt, T., **Jay, GD.**, Elsaid, K. Lubricin/Proteoglycan 4 (PRG4) Binding to CD44 Receptor: A Potential Mechanism Underlying Lubricin's Ability to Suppress Pro-inflammatory Cytokine Induced Synovocyte Proliferation. *Arthritis and Rheumatology*. DOI: 10.1002/art.39087
5. Teeple E., Aslani K., Shalvoy MR., Brody J., Zhang L., Machan J., **Fleming BC., Jay GD.** Lubricin Deficiency in the Murine Lumbar Intervertebral Disc Results in Elevated Torsional Apparent Modulus. *Journal of Biomechanics*. 2015 Apr 3. pii: S0021-9290(15)00201-8 [PMID: 25907550]

6. Sun, Y., Amadio, P., An, K., Zhao, C., **Jay, GD.**, Schmid, T., Yulong, S. Lubricin in Human Achilles Tendon: Shear Force in Achilles Tendon and an Etiology of Achilles Tendinopathy. *Journal of Orthopedic Research*. [J Orthop Res](#). 2015 Jun;33(6):932-7 [PMID: 25864860]
7. Reisdorf, RL., Thoreson, AR., Berglund, LR., Moran, SL., An, K., Amadio, PC., **Jay, GD.** Surface Modification with Chemically Modified Synovial Fluid for Flexor Tendon Reconstruction in a Canine Model in Vivo. *Journal of Bone and Joint Surgery*. (Accepted February 2015) *J Bone Joint Surg Am*. 2015 Jun 17;97(12):972-8 [PMID: 26085530] [PMCID: PMC4469787]
8. Hill, A., Waller, KA., Cui, Y., Allen, JM., Smits, P., Zhang, LX., Ayturk UG., Hann S, Lessard SG., Zurakowski D, Warman, ML., **Jay, GD.** Lubricin Restoration in a Mouse Model of Congenital Deficiency. *Arthritis and Rheumatology*. DOI: 10.1002/art.39276
9. Abubacker, S., Dorosz, SG., Ponjevic, D., Matyas, J., **Jay, GD.**, Schmidt, TA. Cartilage Full-Length Recombinant Human Proteoglycan 4 Interacts with Hyaluronan to Provide Cartilage Boundary Lubrication. *Annals of Biomedical Engineering*. [PMID: 26194040]

#### **Invited Presentations**

1. **Jay, GD:** Tribosupplementation: The Point and Biological Rationale. International Symposium on Intraarticular Treatment. Budapest, October 3rd 2015. [Invited]

#### **Abstracts**

1. Dorosz, S., Abubacker, S., Masala, N., **Jay, G.**, Schmidt TA. Cartilage Boundary Lubricating Ability of Full-Length Human Recombinant PRG4 – Alone and in Combination with Hyaluronan. March 28-31, 2015. Las Vegas, NV.
2. Al-Sharif A., Schmidt T., **Jay G.**, Elsaid K. Lubricin/Proteoglycan-4 Inhibits Pro-inflammatory Cytokine Induced Synoviocyte Proliferation via CD44-Interaction. Orthopedic Research Society. March 28-31, 2015. Las Vegas, NV.
3. Jamal M., Schmidt T., **Jay G.**, Elsaid K. Lubricin/Proteoglycan-4 Binding to Cluster Determinant-44 (CD 44) Receptor and The Contribution of Central Mucin Domain Glycosylations. Orthopedic Research Society. March 28-31, 2015. Las Vegas, NV.
4. Larson, K., Elsaid, K., Schmidt, T., **Fleming, B., Jay, GD.** Tribology of IL-1 Stimulated Cartilage Explants: Restoration of Chondroprotection by rhPRG4. Military Health Research Symposium. August 18-21, 2015, Ft. Lauderdale, FL.
5. Waller KA, Teeple E, McAllister SC, Schmidt TA, **Jay GD, Fleming BC:** Intra-articular rhPRG4 Mitigates Cartilage Damage Following DMM in a Porcine Model. Orthopaedic Research Society, Orlando, 5-8 March 2016.
6. Larson K, Elsaid K, Schmidt T, **Fleming BC, Jay GD:** Restoration of PRG4 mRNA and Chondroprotection by rhPRG4 in IL-1 $\alpha$  Stimulated Cartilage Explants. Orthopaedic Research Society, Orlando, 5-8 March 2016.

7. Waller K, Zhang L, Teeple E, McAllister S, Schmidt T, **Fleming BC, Jay GD**: Recombinant Lubricin Reduces Joint Damage and Inflammation Following Traumatic Injury. Osteoarthritis Research Society International, Amsterdam, 30 March – 3 April 2016.

### **License**

US Patent # 6,743,774 published in 2005 which describes PRG4 and its intended uses has been licensed by Rhode Island Hospital to Lubris, LLC (Framingham, MA). Lubris, LLC is a technology development start up focused on finding therapeutic uses for rhPRG4. A subsequent sublicense is being negotiated with a larger more established biopharma interest to further develop the tribosupplementation technology for traumatized joints in the prevention of post traumatic osteoarthritis.

### **CONCLUSIONS**

Full-length rhPRG4 is the lubricant of choice in restoring chondroprotection to articular cartilage, resulting in cartilage preservation in a large animal model. This is also the case for inflamed cartilage *in vitro* which has been stimulated with IL-1. Histology for IL-1 $\alpha$  treated explants displayed decreased PRG4 expression, which was restituted upon mechanical testing with rhPRG4 as a lubricant. Thus, rhPRG4 is a potential therapeutic in the prevention of OA following joint trauma and in inflamed joints predisposed to OA based on other risk factors. Full-length rhPRG4 can be produced in a scalable production platform using CHO-M cells (Selexis).

### **REFERENCES**

- 1) Al-Shari, A., Jamal, M., Zhang, L., Larson, K., Waller, K., Schmidt, T., **Jay, GD.**, Elsaid, K. Lubricin/Proteoglycan 4 (PRG4) Binding to CD44 Receptor: A Potential Mechanism Underlying Lubricin's Ability to Suppress *Pro*-inflammatory Cytokine Induced Synoviocyte Proliferation. *Arthritis and Rheumatology*. DOI: 10.1002/art.39087